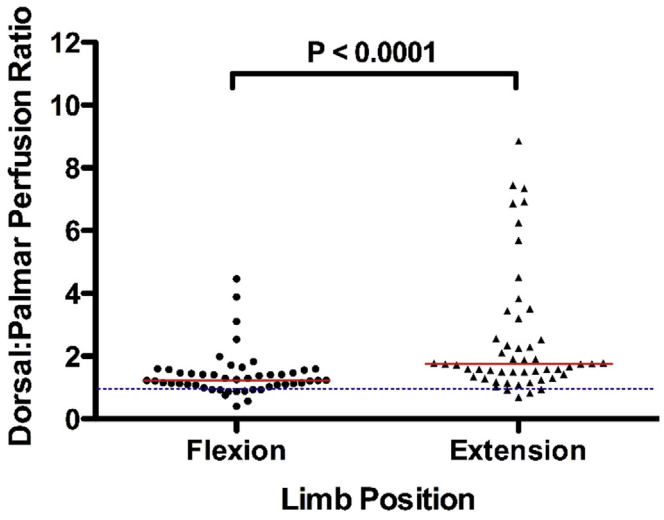

Fig 2. Scatter plot of dorsal:palmar perfusion ratios for flexed and extended fetlock joints. Solid lines indicate median values for the groups, broken line indicates the ratio cutoff point of 1. *** $P < 0.0001$.

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THE RXR AGONIST SR 11237 CAUSES DISTURBED SKELETAL MORPHOGENESIS IN A RAT MODEL

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Introduction: Retinoid X Receptor (RXR) is a type 2 nuclear receptor with important roles in cell death, development, metabolism and cell differentiation. RXR has the ability to form homodimers or heterodimers with other nuclear receptors (PPARs, VDR, LXL and others) in a permissive or non-permissive way. In mice, the non-permissive binding of RXR to the retinoic acid receptor (RAR) has been shown to be important in bone development (exposure to retinoic acid during embryonic development causes deletions and truncations in the forelimbs). However, although the impact of RXR itself is far-reaching, little is known about its relevance in long bone development. To determine its importance, the RXR agonist SR 11237 was used to identify the effects of RXR activation on endochondral ossification, a process through which long bones form by replacing a cartilaginous scaffold with bone.
Purpose: To examine the effects of RXR specific activation on endochondral bone development in the rat.
Methods: Sprague-Dawley rats were given an intraperitoneal injection (IP) of SR 11237 (pan-RXR specific agonist at a concentration of 25 mg per kg) or DMSO (vehicle) once a day from post-natal day 5 to 15. The animals were harvested on post-natal day 16 for Micro-computed Tomography (uCT) scanning (males only), histology and weight determination. For histology, the sections were stained with Safranin O / Fast green, picro-sirius red, Tartrate-resistant acid phosphatase (TRAP) and TUNEL. Further, p57 and SOX9 immunohistochemistry was completed on the sections. For uCT, the limbs were scanned at a resolution of 50 microns / voxel.
Results: RXR activation by SR 11237 causes disturbed ossification and bone morphology in a rat model. uCT analyses and Safranin O staining of the rat long bones show premature growth plate closure and an infiltration of ossified tissue through the central epiphysis of the bone. In addition, the cells surrounding this invasion of ossified material are pre-hypertrophic in size and shape. This is consistent with the pattern of the p57 immunohistochemical staining. SOX9 (a proliferative marker) is also found in the cells surrounding the calcified tissue. In the RXR treated bones, the central epiphysis is highly positive for TRAP staining (an indicator for osteoclastic activity), and picro-sirius red staining reveals a large amount of collagen present. Further, TUNEL staining displays concentrated cell death at the osteo-chondral junction. Although this morphological disorganization is observed in all long bones, uCT also reveals a less calcified, pitted surface on the face of the scapula. The smaller bones of the hands and feet are also thinner and appear somewhat osteopenic. Males are more impaired than females, and the tibia is significantly more afflicted than the humerus and femur. The RXR treated animals of both sexes weigh 30% less than the controls.
Conclusion: In a rat model, increased RXR signaling causes irregular ossification and premature closure of the growth plate. Consequently, this may result in long term effects on long bone and joint morphology, and lead to additional pathology (ex. Osteoarthritis) through joint malalignment or disruption of normal gait.

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OPTIMAL RATIO OF ADIPOSE STEM CELLS AND BONE MARROW STEM CELL TO PROMOTE OSTEOGENIC DIFFERENTIATION AND ANGIOGENESIS

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Purpose: The purpose of this study was to determine the synergistic effect of ASCs added to BMSCs for osteogenic differentiation and angiogenesis, and to find the optimal ratio of ASCs to BMSCs to promote these desired functions.
Methods: In this study, we used transwell and mixed cocultures for the in vitro model and the subcutaneous ectopic ossification in nude mice for the in vivo model. *In vitro* segregated cocultures using transwell were carried out for 14 days in 4 ways using BMSCs and ASCs in passage 3 : #1, 1 x 10^5 BMSCs and 0.25 x 10^5 ASCs; #2, 1 x 10^5 BMSCs and 0.5 x 10^5 ASCs; #3, 1 x 10^5 BMSCs and 0.75 x 10^5 ASCs; #4, 1 x 10^5 BMSCs and 1 x 10^5 ASCs. *In vitro* mixed cocultures were also performed in the same proportion. For *in vivo* implantation of PLGA-ASCs-BMSCs hybrids to evaluate angiogenesis, cells were seeded in PLGA scaffold and implanted on the subcutaneous tissue of 20 nude mice in 5 ways and analyzed after 5 weeks: 1) PLGA scaffold without seeded cells; 2) PLGA seeded with 1 $\times$ 10^5 BMSCs; 3) PLGA seeded with 1 $\times$ 10^5 BMSCs and 0.5 $\times$ 10^5 ASCs; 4) PLGA seeded with 0.67 $\times$ 10^5 BMSCs and 0.33 $\times$ 10^5 ASCs; and 5) PLGA seeded with 1 $\times$ 10^5 ASCs. In critical size calvarial detect model, two circular defects, each with a diameter of 4 mm, were created using trephine burr on the calvarium of 20 nude mice and analyzed 10 weeks. Each of the two defects in each mice was immediately filled with different graft materials; 1) hydroxyapartite (HA)/tricalcium phosphate (β-TCP) granule without cells; 2) HA/β-TCP seeded with 1 $\times$ 10^5 BMSCs; 3) HA/β-TCP with 1 $\times$ 10^5 BMSCs and 0.5 $\times$ 10^5 ASCs (left side), 0.67 $\times$ 10^5 BMSCs and 0.33 $\times$ 10^5 ASCs (right side); and 4) HA/β-TCP with 1 $\times$ 10^5 ASCs.
Results: From the transwell culture, 1 x 10^5 BMSCs cultured with 5 x 10^5 ASCs showed significantly greater osteogenic differentiation and mineralization as shown by alkaline phosphatase (ALP) activity and calcium deposition than BMSCs alone (Fig. 1). In the mixed coculture model, ASC/BMSC coculture at a ratio of 0.5/1 showed a significantly greater level of ALP activity and calcium deposition as well as greater gene and protein expression of osteogenic markers including COL1A, osteocalcin, and bone sialoprotein compared with BMSCs alone (Fig. 2). The mixed ASC/BMSC coculture at a ratio of 0.5/1 showed the highest level of vascular endothelial growth factor which was significantly greater than BMSCs alone, comparable to ASCs alone. The HUVEC tube formation assay also demonstrated that the mixed ASC/BMSC coculture of 0.5/1 enhanced tube formation to a level similar to ASCs alone. In vivo implantation studies demonstrated that PLGA-ASCs-BMSCs showed a greater amount of CD31-positive microvessel formation than PLGA-BMSCs, comparable to that of PLGA-ASCs. PLGA-BMSCs and PLGA-ASCs-BMSCs had similar degrees of calcification, but were greater than that of PLGA-ASCs. *In vivo* effects of ASCs/BMSCs mixture on orthotopic bone formation, when only ceramic granules were treated to the critical size calvarial defect (the control group), most of created defects were not repaired with bone. The defects treated with ceramic granule plus BMSCs were partially repaired with bone, the repaired area being 3.5-fold greater than in the control defect. The defects treated with ceramic granule plus BMSCs and ASCs were also partially repaired with bone. The repaired areas in the defects treated with 1.5 $\times$ 10^5 total ASCs/BMSCs and with 1.0 x 10^5 total cells were 5.0-fold and 4.5-fold greater respectively than in the control defects. Histological findings showed that calvarial defects treated with ceramic/ASCs/BMSCs (1.5 $\times$ 10^5 or 1.0 $\times$ 10^5 total cells) had thicker regenerated calvarium and better reconstitution of osseous structure than those treated with ceramic/BMSCs or ceramic/ASCs.
Conclusions: ASCs added to BMSCs promoted osteogenesis and angiogenesis with the optimal ASCs/BMSCs ratio of 0.5/1.